Effect of ligand diffusion on occupancy fluctuations of cell-surface receptors

Alexander M. Berezhkovskii[1] and Attila Szabo[2]
[1]*Mathematical and Statistical Computing Laboratory, Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, Maryland 20892, USA*
[2]*Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*

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The role of diffusion in the kinetics of reversible ligand binding to receptors on a cell surface or to a macromolecule with multiple binding sites is considered. A formalism is developed that is based on a Markovian master equation for the distribution function of the number of occupied receptors containing rate constants that depend on the ligand diffusivity. The formalism is used to derive (1) a nonlinear rate equation for the mean number of occupied receptors and (2) an analytical expression for the relaxation time that characterizes the decay of equilibrium fluctuations of the occupancy of the receptors. The relaxation time is shown to depend on the ligand diffusivity and concentration, the number of receptors, the cell radius, and intrinsic association/dissociation rate constants. This result is then used to estimate the accuracy of the ligand concentration measurements by the cell, which, according to the Berg-Purcell model, is related to fluctuations in the receptor occupancy, averaged over a finite interval of time. Specifically, a simple expression (which is exact in the framework of our formalism) is derived for the variance in the measured ligand concentration in the limit of long averaging times. [http://dx.doi.org/10.1063/1.4816105]

I. INTRODUCTION

Thermodynamically independent receptors on the surface of a cell are in fact coupled due to the finite rate of ligand diffusion. Because the binding sites compete for ligands, the rate of binding to one receptor depends on the occupancy of the other receptors. This was first pointed out by Berg and Purcell[1] in their classic paper on chemotaxis. Moreover, they showed that such diffusion-induced interactions had a surprising consequence. Specifically, diffusion places a physical limit on how accurately a cell can determine the concentration of an attractant in its surrounding environment.

Berg and Purcell suggested that the best strategy a cell can use to measure the bulk concentration of a ligand is to determine the occupancy of its receptors, not just at a single instant of time, but averaged over a time interval of duration T. This time-averaged occupancy still fluctuates about the equilibrium occupancy when T is finite. At first sight it appears that the cell can reduce the size of these fluctuations by simply increasing the number N of receptors on its surface (i.e., averaging over both time and receptors). Using clever but heuristic arguments, Berg and Purcell showed that when T is sufficiently large, the variance of the time-averaged occupancy does not vanish as N increases. Rather it reaches a finite value determined by the diffusivity of the ligand, the size of the cell, and the averaging time. This result for the limiting accuracy has recently been rederived in different ways[2,3] that appear to us to still contain some uncontrolled approximations and heuristic elements.

In this paper we present what may be regarded as the most straightforward and simplest approximate approach to this problem that has a firm theoretical foundation. Instead of trying to incorporate the effect of diffusion by modifying the chemical rate equation for the mean occupancy,[4] our starting point is a set of chemical rate equations for the concentrations of cells with different numbers of occupied receptors. These equations are equivalent to the master equation for the distribution function of the number of occupied receptors. We then replace all chemical association rate constants by their diffusion-influenced counterparts obtained using the Smoluchowski-Collins-Kimball[5] theory of irreversible bimolecular reactions. Finally, we obtain the corresponding dissociation rate constants by requiring that the equilibrium properties of the system are independent of the ligand diffusivity, as they must be. In this way, for a cell with N receptors, we obtain $(N + 1)$ rate equations with rate constants that are functions of the diffusion constant of the ligand.

Starting from these equations (discussed in Sec. II), we first derive a nonlinear rate equation for the mean occupancy (Sec. III) and an expression (exact in the framework of our model) for the relaxation time of the equilibrium autocorrelation function of receptor occupancy (Sec. IV). Since the variance of the time-averaged occupancy of the receptors is determined by this autocorrelation function, we use these results in Sec. V to estimate the accuracy of the ligand concentration measurements by the cell.

II. THE MODEL

Consider a cell (macromolecule) containing N identical surface receptors (binding sites) in the presence of ligand at concentration c. We are interested in the probability, $P_n(t)$, that n ligands are bound at time t. If the system of interest

contains many cells, this is proportional to the concentration of cells with n occupied receptors. When ligand diffusion is sufficiently fast so that it can be ignored, we can use the formalism of standard chemical kinetics to find $P_n(t)$. Let α_n be the pseudo first order association rate constant for binding a ligand to a cell with n receptors occupied, which is proportional to c, and β_n be the dissociation rate constant that describes the release of a ligand from a cell with n ligands bound. Then variation of the number of occupied receptors is described by the following kinetic scheme

$$0 \underset{\beta_1}{\overset{\alpha_0}{\rightleftharpoons}} 1 \underset{\beta_2}{\overset{\alpha_1}{\rightleftharpoons}} 2 \ldots N-1 \underset{\beta_N}{\overset{\alpha_{n-1}}{\rightleftharpoons}} N. \tag{2.1}$$

The corresponding set of rate equations (also called a master equation) for $P_n(t)$ are

$$\begin{aligned} \frac{dP_0(t)}{dt} &= -\alpha_0 P_0(t) + \beta_1 P_1(t), \\ \frac{dP_n(t)}{dt} &= \alpha_{n-1} P_{n-1}(t) - (\alpha_n + \beta_n) P_n(t) + \beta_{n+1} P_{n+1}(t), \\ \frac{dP_N(t)}{dt} &= \alpha_{N-1} P_{N-1}(t) - \beta_N P_N(t), \end{aligned} \tag{2.2}$$

where $n = 1, 2, \ldots, N-1$. Equation (2.2) can be written in matrix form $d\mathbf{P}/dt = \mathbf{KP}$, where $\mathbf{K}$ is a tri-diagonal $(N+1) \times (N+1)$ matrix of rate constants. Any quantity of interest can be found in terms of the conditional probability of being in state m at time t given that the system initially was in state n, $G_{mn}(t)$. This, in turn, can be expressed as a matrix exponential $\mathbf{G}(t) = \exp(\mathbf{K}t)$.

Here we assume that the receptors are identical and binding is not cooperative. When ligand diffusion is sufficiently fast, the α's and β's can then be expressed in terms of intrinsic bimolecular association (k_+) and unimolecular dissociation (k_-) rate constants of a cell with a single receptor as

$$\alpha_n = (N-n)k_+ c, \tag{2.3a}$$

$$\beta_n = nk_-. \tag{2.3b}$$

The factors in front of the k's have a simple interpretation. A cell with n ligands bound has $N-n$ empty receptors, so a new ligand can bind in $N-n$ different ways. Similarly, a ligand can dissociate from n occupied sites in n different ways.

The corresponding normalized equilibrium distribution can be found by solving Eq. (2.2) when all time derivatives are zero. The result is that P_n^{eq} is binomial

$$P_n^{eq} = \frac{N!}{n!(N-n)!} \frac{(k_+c)^n (k_-)^{N-n}}{(k_+c + k_-)^N}. \tag{2.4}$$

The average number of ligands bound at equilibrium is

$$\langle n \rangle_{eq} = \sum_{n=0}^{N} n P_n^{eq} = \frac{k_+ c}{k_+ c + k_-} N, \tag{2.5}$$

where we have used the fact that for the binomial distribution, $N!/(n!(N-n)!)p^n(1-p)^{N-n}$, the average value of n is Np. The fractional saturation, $\langle n \rangle_{eq}/N$, is the same as that for a cell with one receptor, as to be expected since the receptors were assumed to be thermodynamically independent. For future references, we note that

$$\langle n^2 \rangle_{eq} = \sum_{n=0}^{N} n^2 P_n^{eq} = \langle n \rangle_{eq} + (1 - 1/N) \langle n \rangle_{eq}^2, \tag{2.6}$$

so that the occupancy variance at equilibrium is

$$\langle \delta n^2 \rangle_{eq} = \langle n^2 \rangle_{eq} - \langle n \rangle_{eq}^2 = k_- k_+ c N / (k_+ c + k_-)^2. \tag{2.7}$$

The average number of ligands bound at time t, $\langle n(t) \rangle$, can be shown to satisfy the rate equation

$$\frac{d\langle n(t) \rangle}{dt} = \frac{d}{dt} \sum_{n=0}^{N} n P_n(t) = k_+ c (N - \langle n(t) \rangle) - k_- \langle n(t) \rangle. \tag{2.8}$$

The solution of this equation is

$$\langle n(t) \rangle = \langle n \rangle_{eq} + (\langle n(0) \rangle - \langle n \rangle_{eq}) e^{-(k_+ c + k_-)t}, \tag{2.9}$$

where $\langle n \rangle_{eq}$ is given in Eq. (2.5). Thus the kinetics is completely characterized by a single relaxation time $(k_+ c + k_-)^{-1}$, which is independent of the number of receptors on the cell surface, as to be expected since the receptors are non-interacting.

Let us now consider how the diffusive motion of ligands around the cell influences the kinetics of ligand binding. The problem of developing a theory of reversible diffusion-influenced reactions that is accurate for all times and concentrations is rather challenging (see Ref. 6 and references therein). The underlying reason is that diffusion makes the problem inherently many-body and non-Markovian. For example, the relaxation to equilibrium is no longer exponential as in Eq. (2.9) but becomes a power law at very long times.[7,8] Here we shall generalize chemical kinetics in the simplest possible way that has a solid theoretical foundation. The basic idea is to modify the rate constants in Eq. (2.3a) in such a way that the equilibrium properties of the system are unaltered. The resulting formalism is satisfactory at intermediate times (the short-time behavior is described by the intrinsic rate constants, and, at very long times, the relaxation is a power law) when the ligand concentration is not too high.

To illustrate the procedure in the simplest context, consider a single spherical receptor of radius R, which is assumed to be uniformly reactive with intrinsic association and dissociation rate constants k_+ and k_-, respectively. Following the classical work of Collins and Kimball (CK),[5] we obtain the diffusion-influenced association rate for an irreversible reaction by calculating the steady-state flux into a sphere of radius R. The steady state concentration of the ligand in the bulk satisfies $\nabla^2 c(r) = 0$, subject to the partially reactive boundary condition on the surface of the sphere, $4\pi R^2 D dc(r)/dr|_{r=R} = k_+ c(R)$, where D is the diffusion constant of the ligand. In this way one finds that

$$k_+^{CK} = \frac{k_D k_+}{k_D + k_+}, \tag{2.10}$$

where

$$k_D = 4\pi D R \tag{2.11}$$

is the classic Smoluchowski result for a perfectly absorbing sphere (i.e., when the reaction is completely diffusion-controlled). Equation (2.10) reduces to $k_+^{CK} = k_+$, as $D \to \infty$

or $k_+ \to 0$, (reaction-controlled limit) and to $k_+^{CK} = k_D$, as $D \to 0$ or $k_+ \to \infty$, (diffusion-controlled limit). To find the corresponding diffusion-influenced dissociation rate constant k_-^{CK}, we use the fact that the equilibrium constant depends only on the thermodynamic parameters of the system and cannot depend on the diffusion constant. Since diffusion cannot influence the equilibrium constant, $k_+^{CK}/k_-^{CK} = k_+/k_-$, and so

$$k_-^{CK} = \frac{k_D k_-}{k_D + k_+}. \tag{2.12}$$

Thus when the effective association rate depends on diffusion, then so must the effective dissociation rate. The bound state dissociates to form a contact pair with intrinsic rate constant k_-. But to be considered truly dissociated, the ligand must diffuse far away from the binding site. The effective dissociation rate constant k_-^{CK} is the product of k_- and the probability that a ligand, initially in contact with the binding site, diffuses away rather than recombine, which is given by $k_D/(k_D + k_+)$.

Now let us generalize Eq. (2.10) and find the association rate constant (α_n) to a spherical cell of radius R that has n ligands bound and $(N - n)$ empty receptors. The receptors are identical, each with an intrinsic association rate constant k_+. In principle, for any arrangement of receptors, the analog of k_+^{CK} could be found by solving the steady state diffusion equation subject to the appropriate mixed boundary conditions. Since this is rather complicated, here we adopt a much simpler approach. If the number of receptors is sufficiently large, and the receptors are uniformly distributed on the surface of the cell, we can, to a reasonable approximation, spread the localized reactivities uniformly over the entire surface of the cell. This procedure, in which non-uniform boundary conditions on the cell surface are replaced by an effective uniform boundary condition, is called "boundary homogenization."[9] In the present context, it appears to have been first used in Ref. 10, where a surface partially covered by perfectly absorbing sites was treated as a uniform partially absorbing surface.

Thus to modify the reaction-controlled α_n in Eq. (2.3a) to include the influence of diffusion, one can simply replace k_+ in Eq. (2.10) by $(N - n)k_+$. This leads to

$$\alpha_n = \frac{k_D(N-n)k_+c}{k_D + (N-n)k_+}. \tag{2.13}$$

To find the dissociation rate constant, we use the fact that the equilibrium constant, α_n/β_{n+1}, between cells with n and $(n + 1)$ ligands bound cannot depend on diffusion, so that

$$\frac{\alpha_n}{\beta_{n+1}} = \frac{P_{n+1}^{eq}}{P_n^{eq}} = \frac{(N-n)k_+c}{(n+1)k_-}. \tag{2.14}$$

Combining this with Eq. (2.13) we obtain

$$\beta_{n+1} = \frac{k_D(n+1)k_-}{k_D + (N-n)k_+}. \tag{2.15}$$

The expressions for α_n and β_{n+1} in Eqs. (2.13) and (2.15) correctly reduce to those in Eqs. (2.3a) and (2.3b) as $D \to \infty$. The equilibrium occupancy distribution is still given by Eq. (2.4) for any D.

In the above we have assumed that the binding to an isolated receptor is reaction-controlled. If each receptor is modeled as a partially absorbing disk of radius b, we can generalize the above formalism by replacing k_+ in Eqs. (2.3a), (2.10), and (2.13) by $4Dbk_+/(4Db + k_+)$ and k_- in Eqs. (2.3b), (2.12), and (2.15) by $4Dbk_-/(4Db + k_+)$.[11] Then in the diffusion-controlled limit, $k_+ \to \infty$, the resulting expression for α_0 (see Eq. (2.13)) reduces to the celebrated Berg-Purcell result $4\pi DRNb/(\pi R + Nb)$.[1] Finally, we should mention that we have used the simplest form of boundary homogenization here. For more sophisticated versions, see Ref. 9 and references therein.

III. TIME-DEPENDENCE OF THE AVERAGE NUMBER OF BOUND LIGANDS

Equation (2.2) with rate constants defined in Eqs. (2.13) and (2.15) completely specify our model. As mentioned previously, any quantity of interest can be found numerically by calculating the matrix exponential of an $(N + 1) \times (N + 1)$ rate matrix. Here we explore how much progress can be made analytically. We start by deriving an approximate nonlinear rate equation for the mean number of occupied receptors $\langle n(t)\rangle$.

Consider a kinetic scheme in Eq. (2.1) with the rate constants in Eqs. (2.13) and (2.15) rewritten as $\alpha_n = (N-n)k_+cf_n$ and $\beta_{n+1} = (n+1)k_-f_n$, where $f_n = k_D/(k_D + (N-n)k_+)$. Define a function $g_n = \sum_{m=0}^{n-1} f_m^{-1}$, $n = 1, 2, ..., N$ and $g_0 = 0$. Multiplying both sides of Eq. (2.2) by g_n and summing over all n, it can be shown that $\langle g_n(t)\rangle = \sum_{n=0}^{N} g_n P_n(t)$ satisfies

$$\frac{d\langle g_n(t)\rangle}{dt} = -(k_+c + k_-)(\langle n(t)\rangle - \langle n\rangle_{eq}), \tag{3.1}$$

where $\langle n(t)\rangle$ is the mean number of occupied receptors at time t and $\langle n\rangle_{eq}$ is the equilibrium number of occupied receptors. When $k_D \to \infty$, $f_n = 1$, $g_n = n$, and this equation reduces to Eq. (2.8). Using the definition of f_n and the identity $\sum_{m=0}^{n-1} m = n(n-1)/2$, it can be shown that

$$\langle g_n(t)\rangle = \left(1 + \frac{Nk_+}{k_D} + \frac{k_+}{2k_D}\right)\langle n(t)\rangle - \frac{k_+}{2k_D}\langle n^2(t)\rangle, \tag{3.2}$$

where $\langle n^2(t)\rangle = \sum_{n=0}^{N} n^2 P_n(t)$ is the second moment of the distribution of the number of occupied receptors at time t.

To obtain a closed differential equation for $\langle n(t)\rangle$, we must approximate $\langle n^2(t)\rangle$ in Eq. (3.2) in terms of $\langle n(t)\rangle$. At equilibrium, $\langle n^2\rangle_{eq}$ is related to $\langle n\rangle_{eq}$ by Eq. (2.6). We now assume that this relation holds at all times

$$\langle n^2(t)\rangle = \langle n(t)\rangle + (1 - 1/N)\langle n(t)\rangle^2. \tag{3.3}$$

It can be shown that this is exact for all times when $k_D \to \infty$ (i.e., kinetically non-interacting receptors) and initially all sites are empty. Using Eqs. (3.2) and (3.3), we find that Eq. (3.1) reduces to a nonlinear rate equation for the average number of occupied receptors,

$$\frac{d\langle n(t)\rangle}{dt} = -\frac{k_D(k_+c + k_-)}{k_D + k_+[N - (1 - 1/N)\langle n(t)\rangle]}(\langle n(t)\rangle - \langle n\rangle_{eq}). \tag{3.4}$$

Aside from the factor $1/N$ in the denominator, this equation is equivalent to that obtained by Goldstein and Dembo[4] using heuristic arguments.

This nonlinear rate equation can be solved analytically. The result is

$$\frac{\langle \delta n(t)\rangle}{\langle \delta n(0)\rangle}\exp\left[-\frac{(1-1/N)k_+(\langle \delta n(t)\rangle - \langle \delta n(0)\rangle)}{k_D(k_+c+k_-)\tau_N}\right] = \exp\left(-\frac{t}{\tau_N}\right), \tag{3.5}$$

where $\langle \delta n(t)\rangle \equiv \langle n(t)\rangle - \langle n\rangle_{eq}$ and the time τ_N is defined by

$$\tau_N = \frac{1}{k_+c+k_-} + \frac{k_+(k_+c+Nk_-)}{k_D(k_+c+k_-)^2}. \tag{3.6}$$

It is interesting to note that if $\langle n(t)\rangle$ in the denominator of Eq. (3.4) is replaced by $\langle n\rangle_{eq}$, then this equation becomes

$$\frac{d\langle n(t)\rangle}{dt} = -\frac{1}{\tau_N}(\langle n(t)\rangle - \langle n\rangle_{eq}). \tag{3.7}$$

Thus τ_N can be interpreted as the relaxation time that describes how the mean number of occupied receptors approaches its equilibrium value. In Sec. IV, we will show that the above expression for τ_N turns out to be exact within the framework of our model.

IV. RELAXATION TIME OF THE NUMBER OF BOUND LIGANDS

The relaxation time is formally defined in terms of the normalized autocorrelation function of the number of occupied receptors as

$$\tau_N = \int_0^\infty \frac{\langle \delta n(t)\delta n(0)\rangle_{eq}}{\langle \delta n^2\rangle_{eq}}dt, \tag{4.1}$$

where $\langle \delta n^2\rangle_{eq}$ is the variance given in Eq. (2.7), $\delta n(t) = n(t) - \langle \delta n\rangle_{eq}$, and

$$\langle \delta n(t)\delta n(0)\rangle_{eq} = \sum_{m,n=0}^{N}(m-\langle m\rangle_{eq})G_{mn}(t)(n-\langle n\rangle_{eq})P_n^{eq}. \tag{4.2}$$

Here $G_{mn}(t)$ is the probability of having m ligands bound at time t given that n ligands were bound initially. It can be obtained from the rate matrix $\mathbf{K}$ that corresponds to the kinetic scheme in Eq. (2.1) as $G_{mn}(t) = (\exp(\mathbf{K}t))_{mn}$. The integrand in Eq. (4.1) decreases from unity to zero as time goes from zero to infinity.

To determine τ_N, we will use an identity (proved in the Appendix), which is valid for any function $f(n)$ when the system dynamics is described by the kinetic scheme in Eq. (2.1),

$$\int_0^\infty \langle \delta f(t)\delta f(0)\rangle_{eq}dt = \sum_{n=1}^{N}\left(\beta_n P_n^{eq}\right)^{-1}\left(\sum_{m=n}^{N}\delta f(m)P_m^{eq}\right)^2, \tag{4.3}$$

where $\delta f(t) = \delta f(n(t)) = f(n(t)) - \langle f\rangle_{eq}$. We have used a special case of this formula previously[12] to determine the mean relaxation time of the equilibrium fluctuations of the population of, say, state i ($f(n) = \delta_{in}$) in a system described by a master equation with nearest-neighbor transitions such as Eq. (2.2). The continuous analog of this equation has been used[13] to determine the variance of the efficiency of the Forster resonance energy transfer obtained from photon bursts of duration T.

To find τ_N defined in Eq. (4.1), we must choose $f(n) = n$. Then $\delta f(n) = \delta n = n - \langle n\rangle_{eq}$, and for P_n^{eq} defined in Eq. (2.4), it can be shown that

$$\sum_{m=n}^{N}\delta m P_m^{eq} = \frac{nk_-}{k_+c+k_-}P_n^{eq}. \tag{4.4}$$

Using this along with the definition of β_n in Eq. (2.15), Eq. (4.3) with $f(n) = n$ can be written as

$$\int_0^\infty \langle \delta n(t)\delta n(0)\rangle_{eq}dt = \sum_{n=1}^{N}\left(\frac{1}{k_-n} + \frac{(N+1)k_+}{k_Dk_-n} - \frac{k_+}{k_Dk_-}\right)\frac{n^2k_-^2}{(k_+c+k_-)^2}P_n^{eq}. \tag{4.5}$$

The remaining sums can be done using Eqs. (2.5) and (2.6). Putting it all together, we finally find that the relaxation time of the occupancy of receptors on the cell surface is

$$\tau_N = \frac{1}{k_+c+k_-} + \frac{k_+(k_+c+Nk_-)}{k_D(k_+c+k_-)^2}, \tag{4.6}$$

which is exact for our model. This formula for the relaxation time is one of the main results of this paper. In the reaction-controlled limit ($k_D \to \infty$), τ_N becomes independent of N and reduces to the relaxation time of a single receptor $(k_+c + k_-)^{-1}$. It is interesting to note that the above result turns out to be the same as that given in Eq. (3.6), which was obtained in a non-rigorous way by using the approximate relationship between $\langle n^2(t)\rangle$ and $\langle n(t)\rangle$ in Eq. (3.3).

Finally, we can use τ_N to construct a single-exponential approximation for the autocorrelation function $\langle \delta n(t)\delta n(0)\rangle_{eq}$, which in general is multiexponential. Specifically

$$\langle \delta n(t)\delta n(0)\rangle_{eq} \approx \langle \delta n^2\rangle_{eq}e^{-t/\tau_N}. \tag{4.7}$$

This is exact both at $t = 0$ and $t = \infty$ and has the exact area as defined in Eq. (4.1). These results will be used in Sec. V to estimate the accuracy of ligand concentration measurements by the cell.

V. ACCURACY OF THE LIGAND CONCENTRATION MEASUREMENTS BY A CELL

According to Berg and Purcell,[1] a cell measures the bulk concentration of a ligand by determining the number of occupied receptors averaged over a time interval of duration T. Let $n(t)$ be the instantaneous number of occupied receptors. At equilibrium, this number fluctuates around $\langle n\rangle_{eq}$. Imagine constructing a long trajectory of the number of occupied receptors, say, by applying the Gillespie algorithm to Eq. (2.2). Now divide this trajectory into bins of duration T, and calculate the average number of occupied receptors in each bin, which we will denote by n_T. Without loss of generality, we can set the time in the beginning of each bin equal to zero, so

that for each bin n_T is

$$n_T = \frac{1}{T}\int_0^T n(t)dt. \tag{5.1}$$

n_T approaches its equilibrium value $\langle n\rangle_{eq}$ as $T\to\infty$. Alternatively, if we average the value of n_T over a sufficiently large number of bins, we again recover $\langle n\rangle_{eq}$.

Suppose we can make only one measurement of n_T. The accuracy of this measurement (i.e., how close it is to $\langle n\rangle_{eq}$) is characterized by the variance of the time-averaged occupancy $\langle \delta n_T^2\rangle_{eq}$, where $\delta n_T = n_T - \langle n\rangle_{eq} = (1/T)\int_0^T \delta n(t)dt$. This variance is related to the autocorrelation function $\langle \delta n(t)\delta n(0)\rangle_{eq}$ by

$$\begin{aligned}\langle \delta n_T^2\rangle_{eq} &= (1/T^2)\int_0^T\int_0^T \langle \delta n(t_1)\delta n(t_2)\rangle_{eq} dt_1 dt_2\\ &= (2/T^2)\int_0^T dt_2\int_0^{t_2}\langle \delta n(t_1)\delta n(t_2)\rangle_{eq} dt_1\\ &= (2/T^2)\int_0^T (T-t)\langle \delta n(t)\delta n(0)\rangle_{eq} dt,\end{aligned} \tag{5.2}$$

where we have used the fact that the equilibrium autocorrelation function $\langle \delta n(t_1)\delta n(t_2)\rangle_{eq}$ depends only on the time difference $|t_2 - t_1|$.

When the averaging time T significantly exceeds the relaxation time τ_N, the above variance simplifies to

$$\begin{aligned}\langle \delta n_T^2\rangle_{eq} &= \frac{2}{T}\int_0^\infty \langle \delta n(t)\delta n(0)\rangle_{eq} dt\\ &= \frac{2\tau_N\langle \delta n^2\rangle_{eq}}{T}, \quad T\gg\tau_N,\end{aligned} \tag{5.3}$$

where we have used the definition of τ_N in Eq. (4.1). Since we have evaluated τ_N exactly within the framework of our model, Eq. (5.3) provides the exact large-T behavior of the variance $\langle \delta n_T^2\rangle_{eq}$. We can get an approximate expression for the variance for all T, by substituting the single-exponential approximation for the autocorrelation function $\langle \delta n(t)\delta n(0)\rangle_{eq}$ in Eq. (4.7) into Eq. (5.2) and evaluating the integral. The result is

$$\left\langle \delta n_T^2\right\rangle_{eq} \approx \frac{2(T/\tau_N - 1 + e^{-T/\tau_N})}{(T/\tau_N)^2}\langle \delta n^2\rangle_{eq}. \tag{5.4}$$

This is exact in both the $T\to 0$ and $T\to\infty$ limits and provides a useful approximation for intermediate bin times.

Following Berg and Purcell,[1] we introduce a time-averaged ligand concentration, c_T, measured by a cell with N receptors on its surface, which is related to the time-averaged receptor occupancy n_T by

$$n_T = \frac{k_+c_T}{k_+c_T + k_-}N \tag{5.5}$$

(cf. Eq. (2.5)). The variance of the concentration c_T is related to the variance of the time-averaged occupancy by

$$\left\langle \delta c_T^2\right\rangle_{eq} = c^2\left\langle \delta n_T^2\right\rangle_{eq}/\langle \delta n^2\rangle_{eq}^2, \tag{5.6}$$

where we have used the fact that the variance of a function $f(x)$ of a random variable x, $\langle \delta f^2\rangle$, is related to the mean and variance of its argument, $\langle x\rangle$ and $\langle \delta x^2\rangle$, by $\langle \delta f^2\rangle = f'(\langle x\rangle)^2\langle \delta x^2\rangle$, where $f'(x) = df(x)/dx$.

Assuming that $T\gg\tau_N$, we find the T-dependence of $\langle \delta c_T^2\rangle_{eq}$ by combining Eqs. (5.3) and (5.6), $\langle \delta c_T^2\rangle_{eq}/c^2 = 2\tau_N/(\langle \delta n^2\rangle_{eq}T)$. Using the expression for τ_N given in Eq. (4.6), we finally obtain one of the key results of this paper, namely that, for large averaging times, the variance of the measured concentration is

$$\frac{\langle \delta c_T^2\rangle_{eq}}{c^2} = \frac{2}{Nk_+cT}\left(1+\frac{k_+c}{k_-}\right) + \frac{1}{2\pi DRcT}\left(1+\frac{k_+c}{Nk_-}\right). \tag{5.7}$$

This shows how the relative variance depends on the averaging time T, the number N of receptors on the cell surface, the cell radius R, ligand concentration c, and diffusion constant D, as well as the intrinsic association and dissociation rate constants k_+ and k_-. The expression in Eq. (5.7) is exact within the framework of our model. The first term is the result predicted by standard chemical kinetics: the variance corresponding to a single receptor, $2(k_+c + k_-)/(k_-k_+cT)$, is reduced by a factor of N, as to be expected for independent receptors. The second term is the result of competition among the receptors for diffusing ligands. In contrast to the first term, it does not vanish, as $N\to\infty$. Thus it places a physical limit on how accurately a cell can determine the concentration of a ligand.

As N increases, Eq. (5.7) approaches

$$\frac{\langle \delta c_T^2\rangle_{eq}}{c^2} = \frac{1}{2\pi DRcT}. \tag{5.8}$$

Thus at sufficiently large T and N, the variance reaches a limiting value, which is independent of N. This expression differs from the result given by Berg and Purcell[1] only by a factor $(1 + k_+c/k_-)$. This is rather remarkable because they evaluated $\langle \delta c_T^2\rangle_{eq}/c^2$ using heuristic arguments.

Our result is also related to the more recent work of Wang *et al.*,[3] who focused on the low concentration limit. Using a combination of physical arguments and stochastic simulations, they obtained an expression for the variance (Eqs. (9) and (11) from Ref. 3), which in the $c\to 0$ limit is the same as our Eq. (5.7) in this limit. It would be interesting to see if their intuitive arguments could be generalized to treat the case of arbitrary ligand concentration.

VI. CONCLUDING REMARKS

The number of occupied receptors on the surface of a cell fluctuates around its equilibrium value. In this paper we study how ligand diffusion affects the decay of equilibrium fluctuations of the receptor occupancy. Main results of our analysis are given in Eqs. (3.4), (4.6), and (5.7). Specifically, the nonlinear rate equation in Eq. (3.4) describes the relaxation to equilibrium of the mean number of occupied receptors. The expression in Eq. (4.6) gives the relaxation time that characterizes the decay of equilibrium fluctuations of the receptor occupancy. These results were obtained in Secs. III and IV using the formalism developed in Sec. II. The formalism is based on a set of ordinary rate equations, Eq. (2.2), for the distribution function of the number of occupied receptors. The association/dissociation rate constants entering into these equations, given in Eqs. (2.13) and (2.15), are

functions of the ligand diffusion constant. We applied the above results to the Berg-Purcell model of the ligand concentration measurements by a cell, which assumes that the cell learns about the concentration of an attractant by determining the time-averaged occupancy of its surface receptors. A simple expression in Eq. (5.7), which is exact in the framework of our model, gives the variance of the concentration measured by the cell when the averaging time is sufficiently long. Our results show how the accuracy of the measurement depends on the number of receptors on the cell surface, the ligand concentration and its diffusivity, intrinsic association/dissociation rate constants, and the cell radius.

The theory presented here can be extended by using the self-consistent relaxation time approximation (SCRTA) for the kinetics of reversible diffusion-influenced reactions.[6] For multiple reaction sites the algebra becomes rather complicated. Therefore, to get a feeling for the nature of the corrections, let us consider the simplest case of a single spherical receptor modeled as a uniformly reactive sphere of radius R that can reversibly bind only one ligand. In such a case, our result in Eq. (5.7) ($N = 1, T \gg \tau_1$) reduces to

$$\frac{\langle \delta c_T^2 \rangle_{eq}}{c^2} = \frac{2}{k_+^{CK} cT}\left(1 + \frac{k_+ c}{k_-}\right), \tag{6.1}$$

where k_+^{CK} is given by Eq. (2.10). When $D \to \infty$, k_+^{CK} becomes equal to k_+, and the above result reduces to one that can be obtained in the framework of chemical kinetics. To obtain the corresponding result within the framework of SCRTA, we note that by Onsager's regression hypothesis the normalized autocorrelation function $\langle \delta n(t)\delta n(0)\rangle_{eq} / \langle \delta n^2 \rangle_{eq}$ is identical to the relaxation function, $R(t)$, which describes relaxation of the receptor occupancy to equilibrium. Using Eqs. (4.11) and (4.15) of Ref. 6 we find that the variance of the measured concentration is given by Eq. (6.1) with k_+^{CK} replaced by k_+^{SCRTA}, which is a solution to

$$\frac{1}{k_+^{SCRTA}} = \frac{1}{k_+^{CK}} - \frac{k_+ c}{k_D(k_+ c + k_-)\{1 + \sqrt{k_+ D/[R^2(k_+ c + k_-)k_+^{SCRTA}]}\}}, \tag{6.2}$$

where k_D is given by Eq. (2.11). The rate constant k_+^{SCRTA} is a function of the ligand concentration. It approaches k_+^{CK} from above as c tends to zero. When the spherical receptor is immobile and the ligands do not interact with each other, Eq. (6.2) is expected to be essentially exact for all c.

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APPENDIX: RELAXATION TIME OF AN AUTOCORRELATION FUNCTION

The relaxation time that describes the decay of the equilibrium fluctuations of a certain quantity is related to the time integral of the autocorrelation function of that quantity (e.g., see Eq. (4.1)). Here we derive Eq. (4.3) by evaluating this integral analytically for any system whose dynamics is described by the kinetic scheme shown in Eq. (2.1). We begin by rewriting the formal expression for the time integral of the autocorrelation function of $\delta f_n = f_n - \langle f \rangle_{eq}$ as

$$\int_0^\infty \langle \delta f(t)\delta f(0)\rangle_{eq} dt = \int_0^\infty \sum_{m,n=0}^{N} \delta f_m G_{mn}(t) \delta f_n P_n^{eq} dt = \sum_{m=0}^{N} \delta f_m v_m, \tag{A1}$$

where we have defined

$$v_m = \sum_{j=0}^{N} \int_0^\infty G_{mj}(t) dt\, \delta f_j P_j^{eq}. \tag{A2}$$

Here P_j^{eq} is the normalized equilibrium population of state j, $\sum_{j=0}^{N} P_j^{eq} = 1$, that can be found using the detailed balance condition $\alpha_{i-1} P_{i-1}^{eq} = \beta_i P_i^{eq}$. The propagator or Green's function $G_{ij}(t)$ satisfies the master equation

$$\frac{d}{dt} G_{ij}(t) = \sum_{m=0}^{N} K_{im} G_{mj}(t), \tag{A3}$$

with initial condition $G_{ij}(0) = \delta_{ij}$. K_{im} is the imth element of a tri-diagonal rate matrix $\mathbf{K}$ that corresponds to the kinetic scheme in Eq. (2.1).

Multiplying both sides of Eq. (A2) by K_{im}, summing over all m, and using Eq. (A3), we find that

$$\sum_{m=0}^{N} K_{im} v_m = \sum_{j=0}^{N} \int_0^\infty \frac{d}{dt} G_{ij}(t) dt\, \delta f_j P_j^{eq}. \tag{A4}$$

Evaluating the time integral using the facts that $G_{ij}(0) = \delta_{ij}$, $G_{ij}(\infty) = P_i^{eq}$, and $\sum_{j=0}^{N} \delta f_j P_j^{eq} = 0$, we obtain

$$\sum_{m=0}^{N} K_{im} v_m = -\delta f_i P_i^{eq} \tag{A5}$$

or explicitly

$$\alpha_{i-1} v_{i-1} - \alpha_i v_i - \beta_i v_i + \beta_{i+1} v_{i+1} = -\delta f_i P_i^{eq}. \tag{A6}$$

Let us now set $\alpha_{-1} = \beta_0 = \alpha_N = \beta_{N+1} = 0$, so that one will not have to worry about the end points in what follows.

Summing both sides of Eq. (A6) from $i = n$ to $i = N$, we find that

$$-\alpha_{n-1} v_{n-1} + \beta_n v_n = \sum_{i=n}^{N} \delta f_i P_i^{eq}. \tag{A7}$$

Now, we introduce a new variable u_n defined as

$$v_n = u_n P_n^{eq}. \tag{A8}$$

Using the detailed balance condition $\alpha_{n-1}P^{eq}_{n-1} = \beta_n P^{eq}_n$, we can write Eq. (A7) as

$$u_n - u_{n-1} = \left(\beta_n P^{eq}_n\right)^{-1} \sum_{i=n}^{N} \delta f_i P^{eq}_i. \tag{A9}$$

Summing both sides from $n = 1$ to $n = m$, we obtain

$$u_m = u_0 + \sum_{n=1}^{m} \left(\beta_n P^{eq}_n\right)^{-1} \sum_{i=n}^{N} \delta f_i P^{eq}_i. \tag{A10}$$

We use this to find v_m from Eq. (A8). Substituting the result into Eq. (A1), we arrive at

$$\int_0^\infty \langle \delta f(t)\delta f(0)\rangle_{eq} dt = \sum_{m=1}^{N} \delta f_m P^{eq}_m \sum_{n=1}^{m} \left(\beta_n P^{eq}_n\right)^{-1} \sum_{i=n}^{N} \delta f_i P^{eq}_i, \tag{A11}$$

where we have used the fact that $\sum_{m=0}^{N} \delta f_m P^{eq}_m = 0$. By interchanging the order of summation, we recover Eq. (4.3).

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